Journal of Microbiology (2019) Vol. 57, No. 4, pp. 238–242
DOI 10.1007/s12275-019-8590-0
eISSN 1976-3794
pISSN 1225-8873

Mesorhizobium denitrificans sp. nov., a novel denitrifying bacterium isolated from sludge[§]

Muhammad Zubair Siddiqi[1,2], Ngo Thi Phuong Thao[3], Gyumin Choi[2], Dae-Cheol Kim[1], Young-Woo Lee[1], Sang Young Kim[4], Ji-Hyang Wee[4], and Wan-Taek Im[1,2]*

[1]*Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea*
[2]*AceEMzyme Co., Ltd., Academic Industry Cooperation, Anseong 17579, Republic of Korea*
[3]*M-ChET, KIOCA program, Hankyong National University, Anseong 17579, Republic of Korea*
[4]*Department of Food Science & Bio Technology, Shinansan University, Ansan 15435, Republic of Korea*

(Received Oct 25, 2018 / Revised Nov 22, 2018 / Accepted Nov 22, 2018)

A Gram-stain-negative, non-spore-forming, facultative, rod-shaped bacterium (designated LA-28^T) was isolated from a sludge sample from a wastewater treatment plant in Hanam city, Republic of Korea. On the basis of 16S rRNA gene sequencing, strain LA-28^T clustered with species of the genus *Mesorhizobium* and appeared closely related to *M. jarvisii* LMG 28313^T (96.8%), *M. waimense* ICMP 19557^T (96.7%), and *M. huakuii* LMG 14107^T (96.7%). Growth occurs at 18–40°C on R2A medium in the presence of 1–4% NaCl (w/v) and at pH 6–8. The DNA G+C content was 61.2 mol%, and the predominant quinone was ubiquinone-10 (Q-10). The major cellular fatty acids (> 5%) were $C_{16:0}$, $C_{19:0}$ *ω8c* cyclo, $C_{18:1}$ *ω7c* 11-methyl, and $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c* (summed feature 8). Major polar lipids were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidyl-N-methylethanolamine (PME), and phosphatidylcholine (PC). Physiological and biochemical characteristics indicated that strain LA-28^T represents a novel species of the genus *Mesorhizobium*, for which the name *Mesorhizobium denitrificans* sp. nov. is proposed. The type strain is LA-28^T (= KACC 19675^T = LMG 30806^T).

Keywords: *Mesorhizobium denitrificans*, 16S rRNA gene sequence, polyphasic taxomony, sludge

*For correspondence. E-mail: wandra@hknu.ac.kr; Tel.: +82-31-6705335; Fax: +82-31-6705339
[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

Introduction

In a study of the microbial community and denitrifying bacteria in the sludge from a wastewater treatment plant in Hanam city (37°32′44.4″ N, 127°13′05.4″ E), Republic of Korea, a novel bacterium (strain LA-28^T) was isolated. The 16S rRNA and phylogenetic analysis assigned the strain LA-28^T to the phylum *Proteobacteria*, class *Alphaproteobacteria*, order *Rhizobiales*, family *Phyllobacteriaceae*, and genus *Mesorhizobium*. The genus *Mesorhizobium* was first proposed by Jarvis *et al.* (1997), who suggested moving the five *Rhizobium* species to *Mesorhizobium* gen. nov. At the time of this writing, the genus contains more than 49 species (http://www.bacterio.net), including *Mesorhizobium calcicola* and *Mesorhizobium kowhaii* (De Meyer *et al.*, 2016), *Mesorhizobium japonicum* (Martinez-Hidalgo *et al.*, 2016), *Mesorhizobium sediminum* (Yuan *et al.*, 2016), and *Mesorhizobium hankyongi* (Siddiqi *et al.*, 2018).

Materials and Methods

Bacterial strains isolation

To screen for denitrifying bacterial strains living in sludge from a wastewater treatment plant in Hanam city, sludge samples were collected from different places in the wastewater treatment plant and transferred to a laboratory for isolation of denitrifying bacterial strains. The samples were carefully suspended in 0.85% saline and spread on R2A agar medium (Difco) plates. Then, the plates were incubated at 30°C for 2 weeks. After 2 weeks, the strains were purified by subculturing on new R2A agar plates. Strain LA-28^T was routinely cultured on fresh R2A agar and maintained in a glycerol suspension (R2A broth with 20%, v/v), at -80°C.

In this current report, we describe a novel bacterial strain, designated LA-28^T, which appears to be a member of the genus *Mesorhizobium*. Reference strains (*M. jarvis* LMG 28313^T and *M. huakuii* LMG 14107^T) were obtained from Belgian Coordinated Collections of Microorganisms (BCCM/LMG Bacteria Collection) for use in a comparative analysis.

Physiological and biochemical analysis

The Gram-stain type was determined using the described method of Buck in 1982 (Buck, 1982). Strain morphology was examined by transmission electron microscope (Hitachi SU-3500), after cells grown for 2 days at 30°C on R2A agar medium. Motility was checked by hanging drop method. Tests for degradation of casein, Tween 80, Tween 20, starch, and DNA were evaluated after 3 days of incubation at 30°C (Atlas,

1993). Catalase and oxidase activities were determined as previously described (Cappuccino and Sherman, 2002). Biochemical tests were carried out using commercial API (API ID 32GN, API 20NE, and API ZYM) kits according to the manufacturer (bioMérieux) instructions. The API ZYM test strip was read after 4 h of incubation at 37°C, and the other API strips were examined after 2 days at 30°C. Growth at various temperatures (4–45°C at interval 5°C) and various pH values (pH 3.5–10.0 at intervals of 0.5 and 1.0 pH units) was assessed after 7 days of incubation at 30°C using R2A broth medium. The following buffers (each 20 mM final concentration) were used to adjust the pH of R2A broth: acetate buffer for pH 3.5–5.5, phosphate buffer for pH 6.0–8.0 and Tris buffer for pH 8.5–10.0. Salinity tolerance test was performed on R2A agar medium supplemented with 0–10% (w/v at intervals of 1% unit) NaCl after 7 days of incubation at 30°C. Growth on different media [nutrient agar (NA, Difco), R2A agar (Difco), Luria-Bertani (Difco), DNAse agar (Difco), MacConkey, and TSA agar (Difco)] was evaluated after 5 days of incubation at 30°C. Nitrate reduction ability was analyzed by inoculating the strain in R2A broth supplemented with two different concentrations of KNO_3 (100 ppm and 1,000 ppm) and incubated at 30°C in aerobic and anaerobic conditions. For 4 days at 12-h intervals, samples were withdrawn, cell growth was measured by spectrophotometer, and nitrate reduction was analyzed using a HS-NO_3 (N)-CA kit.

Phylogenetic analysis

Genomic DNA of strain LA-28^T was isolated using a genomic DNA extraction kit (Solgent Co. Ltd.) and the 16S rRNA gene was amplified using the universal bacterial primer set (800R, 1492R, 27F, and 518F) (Lane, 1991). Then, the purified PCR products were sequenced by Solgent Co. Ltd. Almost full-length sequence of the 16S rRNA gene was compiled and the 16S rRNA gene sequences of related taxa were obtained from the EzTaxon-e server and GenBank database. Multiple sequence alignments were performed by Clustal_X program with gaps edited in BioEdit program (Thompson *et al*., 1997; Hall, 1999). Evolutionary distances were calculated through Kimura two-parameter model and the phylogenic trees were constructed with neighbor-joining (Kimura, 1983; Saitou and Nei, 1987), maximum-likelihood and maximum-parsimony (Fitch, 1971) algorithms by using MEGA 6 Program (Tamura *et al*., 2013) with 1,000 replications (Felsenstein, 1985).

DNA G+C content (mol%)

For the measurement of DNA G+C content, genomic DNA of the novel strain was extracted and purified as described by Moore and Dowhan (1995) and was enzymatically degraded into nucleosides, and was determined as described before (Mesbah *et al*., 1989) using a reverse-phase HPLC.

Chemotaxonomic analysis

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions, and reextracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analyzed by HPLC as previously described (Hiraishi *et al*., 1996). Cellular fatty acids profiles were determined for strains grown on R2A agar for 48 h. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analysed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Strain LA-28^T was examined for their polar lipid contents as described previously (Minnikin *et al*., 1984).

Draft genome sequence analysis

A draft genome sequence of the novel isolate (LA-28^T) was obtained by Illumina HiSeq X Ten analysis and assembled using the SOAPdenovo v. 3.10.1 *de novo* assembler. Using the NCBI prokaryotic genome annotation pipeline (PGAP), the draft genome was annotated.

Nucleotide sequence accession numbers

The draft genome and 16S rRNA gene sequences of strain LA-28^T has been deposited at GenBank/EMBL/DDBJ under accession numbers QURN00000000 and MH209624, respectively.

Digital protologue number

The digital protologue number of strain LA-28^T is TA00717.

Results and Discussion

Morphological and phenotypic characteristics

Colonies of strain LA-28^T grown on R2A agar plates for 2 days at 30°C were convex, milky colored. Cells were non-motile and rod-shaped (0.5–1.0 μm in diameter and 1.5–2.6 μm in length) (Fig. 1). Negative for hydrolyses of starch, casein, Tween 80, Tween 20, and DNA. Strain LA-28^T was able to grow at 18–40°C, but not grow bellow 18 and above 40°C. The novel isolate was positive for the reduction of nitrate to nitrite. Furthermore, the physiological and biochemical

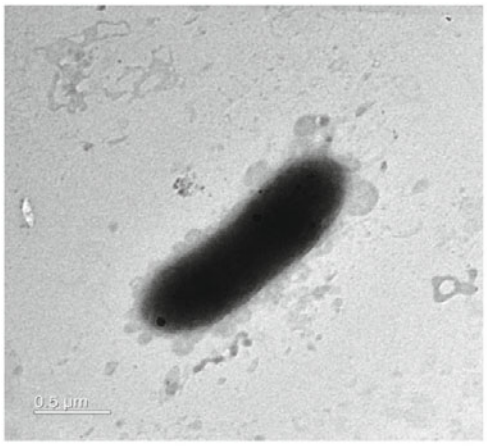

Fig. 1. Transmission electron micrograph of strain LA-28^T. Bar, 5 μm.

Table 1. Physiological and biochemical characteristics between strain LA-28^T and closely related species of the genus *Mesorhizobium*
1, LA-28^T; 2, *M. jarvisii* LMG 28313^T; 3, *M. huakuii* LMG 14107^T. All tests were obtained in this study.
All strains are positive for urease, esculin hydrolysis, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase, α-glucosidase, D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, L-histidine, 3-Hydroxy-butyrate, L-rhamnose, N-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, acetate, lactate, L-alanine. Negative for nitrate reduction, indole production, lipase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, glucose acidification, gelatin hydrolysis, gluconate, caprate, adipate, citrate, phenyl-acetate, 4-hydroxy-benzoate, itaconate, malonate, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine. +, positive; -, negative.

Characteristics	1	2	3
Temperature range for growth (°C)	18–40	10–38[a]	ND
pH range for growth	6.0–8.0	5.0–8.0[a]	5–9.5[b]
NaCl tolerance (% NaCl, w/v) range for growth	0.5–4.0	0.5–2.0[a]	0.5–1.0[b]
Enzymes activities for:			
Arginine dihydrolase	+	-	-
Cystine arylamidase	-	+	+
β-Galactosidase (PNPG)	-	+	-
Naphtol-AS-BI-phosphohydrolase	+	-	-
Trypsin	+	-	-
Valine arylamidase	-	+	-
Utilization of:			
Glycogen	+	-	-
D-Glucose	+	+	-
2-Ketogluconate	+	+	-
Malate	-	+	+
Propionate	-	+	-
L-Proline	+	-	+
Suberate	+	-	-
Valerate	+	-	-
DNA G+C content (mol%)	61.2	62.7[a]	59-64[b]

[a, b] data taken from: [a] Martinez-Hidalgo *et al.* (2016), [b] Chen *et al.* (1991), and Velázquez *et al.* (2001).

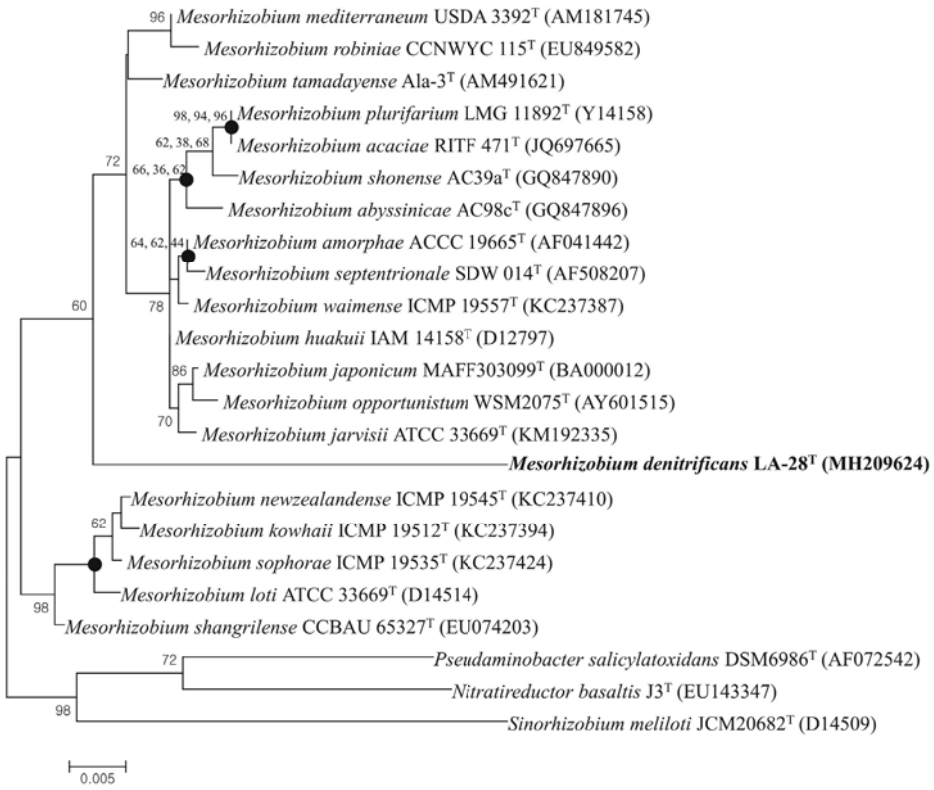

Fig. 2. Phylogenetic tree showing the relationships of strain LA-28^T with other related species of the genus *Mesorhizobium*. The tree was made using the maximum-likelihood method. Dots circles at the nodes indicate that generic branches were also recovered by using neighbor-joining and maximum-parsimony algorithms. Bootstrap values expressed as percentages of 1,000 replications) greater than 60% are shown at the branch points. Bar, 0.005 substitutions per nucleotide position.

characteristics of strain LA-28^T are summarized in the description and Table 1.

Phylogenetic and DNA G+C content analysis

An almost complete 16S rRNA gene sequence of strain LA-28^T was assembled using SeqMan software (DNASTAR), and the sequence was compared with the 16S rRNA gene sequences of several other bacterial taxa, which were obtained from the EzBioCloud server [http://www.ezbiocloud.net/eztaxon] and GenBank database. The novel isolate was found to belong to the genus *Mesorhizobium* and show highest sequence similarity to *M. jarvisii* LMG 28313^T (96.8%) and *M. alhagi* LMG 28228^T (96.7%). Based on the neighbor-joining, maximum-likelihood, and maximum-parsimony algorithms, strain LA-28^T clustered with sequences from bacteria in the genus *Mesorhizobium* (Fig. 2) and formed a clade with *M. jarvisii* LMG 28313^T (Supplementary data Fig. S1). The DNA G+C Content of strain LA-28^T was 62.1 mol%.

Chemotaxonomic characteristics

The major quinone detected in strain LA-28^T was ubiquinone-10 (Q-10), which is same to other species in genus *Mesorhizobium*. The major cellular fatty acids of strain LA-28^T were mainly composed of $C_{16:0}$ (10.0%), $C_{18:1}$ *ω7c* 11-methyl (9.2%), and summed feature 8 [comprising $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c* (60.9%)], which is similar to those of described species in the genus *Mesorhizobium* (Table 2). The major polar lipids were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidyl-N-methylethanolamine (PME), and phosphatidylcholine (PC). The minor lipid was one unidentified phospholipid (PL) (Fig. 3). From the polar lipid analysis, the novel isolate was found to share major polar lipids PG, PE, PME, and PC with described species in the genus *Mesorhizobium* (Yuan *et al*., 2016; Siddiqi *et al*., 2018).

Table 2. Fatty acid profiles of strain LA-28^T and related species of the genus *Mesorhizobium*

1, LA-28^T; 2, *M. jarvisii* LMG 28313^T; 3, *M. huakuii* LMG 14107^T.
All strains were cultured on R2A agar medium for 48 h at 30°C. Fatty acids amounting to < 0.5% of the total fatty acids in all strains are not listed. tr, trace amounting (tr > 0.5%); –, not detected.

Fatty acids	1	2	3
Saturated			
$C_{16:0}$	10.0	7.0	9.3
$C_{17:0}$	2.5	1.6	1.4
$C_{18:0}$	4.1	4.3	6.1
$C_{20:0}$	-	1.0	0.9
Branched-chain fatty acid			
iso-$C_{15:0}$	3.8	-	-
iso-$C_{17:0}$	-	5.1	5.9
$C_{19:0}$ *ω8c* cyclo	8.9	13.1	19.7
Unsaturated			
$C_{17:1}$ *ω8c*	-	1.2	0.9
$C_{18:1}$ *ω7c* 11-methyl	9.2	15.9	10.7
Summed feature*			
8; $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*	60.9	50.5	44.4

*Summed features represent groups of two or three fatty acids that could not be separated by gas chromatography (GLC) with the MIDI system.

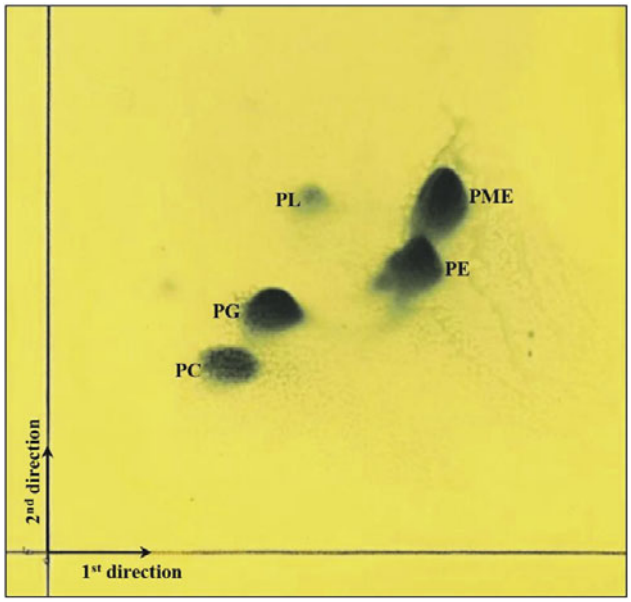

Fig. 3. Two-dimensional TLC of the total polar lipids of strain LA-28^T. TLC plates were stained for total polar lipids with 5% ethanolic molybdophosphoric acid. Abbreviations: PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PME, phosphatidyl-N-methylethanolamine; PC, phosphatidylcholine; L, unidentified polar lipid.

Taxonomic conclusions

Based on our taxonomic and morphological analyses, strain LA-28^T shares major ubiquinone Q-10, $C_{16:0}$, $C_{18:1}$ *ω7c* 11-methyl, and summed feature 8 as major fatty acids (CFAs) and phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidyl-N-methylethanolamine (PME), and phosphatidylcholine (PC) as major polar lipids with described species in the genus *Mesorhizobium*. However, on the basis of its phylogenetic distance from known *Mesorhizobium* species, high amounts of summed feature 8 and low amounts of cyclo-$C_{19:0}$ *ω8c*, and a combination of unique phenotypic characteristics (as shown in Table 1), strain LA-28^T represents a novel species in the genus *Mesorhizobium*. The name *Mesorhizobium denitrificans* sp. nov. is proposed for this new species.

Description of *Mesorhizobium denitrificans* sp. nov.

Mesorhizobium denitrificans (de.ni.tri′fi.cans. N.L. v. *denitrifico* to denitrify; N.L. part. adj. *denitrificans* denitrifying).

Cells are facultative anaerobic, oxidase negative, and catalase positive. Colonies grown on R2A agar are opaque, circular, and milky coloured. Growth occurs at 18–40°C in the presence of 1–4% NaCl (w/v) and at pH 6–8. Optimum growth occurs at 30°C and pH 6.5–7.0 in the absence of NaCl. In both aerobic and anaerobic condition it reduce nitrate to nitrite. Negative for the hydrolysis of casein, DNase, starch, Tween 80, and Tween 20. The strain grow well on R2A agar medium, whereas weakly grow on TSA, NA, and LB agar media, but did not grow on DNAse agar and MacConkey agar. In commercial API (ZYM, 20NE, and 32GN) kits positive for arginine dihydrolase, urease, esculin hydrolysis, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase,

trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, L-fucose, D-sorbitol, L-arabinose, valerate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, L-proline, L-rhamnose, N-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, suberate, acetate, L-lactate, alanine, and glycogen. Negative for indole production, glucose acidification, gelatin hydrolysis, α-galactosidase, β-galactosidase, β-glucuronidase, lipase, valine arylamidase, cystine arylamidase, α-chymotrypsin, gluconate, caprate, adipate, malate, citrate, phenyl-acetate, salicin, D-melibiose, propionate, caprate, 4-hydroxy-benzoate, itaconate, malonate, 5-ketogluconate, 3-hydroxy-benzoate, L-serine, α-mannosidase, and α-fucosidase. The predominant quinone is Q-10. The major cellular fatty acids are $C_{16:0}$, $C_{19:0}$ cyclo *ω8c*, and summed feature 8. The polar lipids are PG, PE, PME, and PC, and one unidentified phospholipid PL. The DNA G+C content of genomic DNA is 61.2 mol%.

The type strain, LA-28^T (= KACC 19675^T = LMG 30806^T) was isolated from the sludge of wastewater treatment plant Hanam city, South Korea.

Acknowledgements

This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) and by the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ012283)" Rural Development Administration, Republic of Korea, and by a grant from the Korea Research Institute of Bioscience & Biotechnology (KRIBB) Research Initiative Program.

References

Atlas, R.M. 1993. Handbook of microbiological media. CRC Press, Boca Raton, Florida, USA.

Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* **44**, 992–993.

Cappuccino, J.G. and Sherman, N. 2002. Microbiology. a laboratory manual, 6th ed. Pearson Education, Inc., California, USA.

Chen, W.X., Li, G.S., Qi, Y.L., Wang, E.T., Yuan, H.L., and Li, J.L. 1991. *Rhizobium huakuii* sp. nov. isolated from the root nodules of *Astragalus sinicus*. *Int. J. Syst. Bacteriol.* **41**, 275–280.

De Meyer, S.E., Tan, H.W., Andrews, M., Heenan, P.B., and Willems, A. 2016. *Mesorhizobium calcicola* sp. nov., *Mesorhizobium waitakense* sp. nov., *Mesorhizobium sophorae* sp. nov., *Mesorhizobium newzealandense* sp. nov. and *Mesorhizobium kowhaii* sp. nov. isolated from *Sophora* root nodules. *Int. J. Syst. Evol. Microbiol.* **66**, 786–795.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* **20**, 406–416.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.

Hiraishi, A., Ueda, Y., Ishihara, J., and Mori, T. 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* **42**, 457–469.

Jarvis, B.D.W., Van Berkum, P., Chen, W.X., Nour, S.M., Fernandez, M.P., Cleyet-Marel, J.C., and Gillis, M. 1997. Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. *Int. J. Syst. Bacteriol.* **47**, 895–898.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge, UK.

Lane, D.J. 1991. 16S/23S rRNA sequencing. *In* Stackebrandt, E. and Goodfellow, M. (eds.), Nucleic acid techniques in bacterial systematics. Wiley, New York, USA.

Martinez-Hidalgo, P., Ramirez-Bahena, M.H., Flores-Felix, J.D., Igual, J.M., Sanjuan, J., Leon-Barrios, M., Peix, A., and Velazquez, E. 2016. Reclassification of strains MAFF 303099^T and R7A into *Mesorhizobium japonicum* sp. nov. *Int. J. Syst. Evol. Microbiol.* **66**, 4936–4941.

Mesbah, M., Premachandran, U., and Whitman, W.B. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**, 159–167.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Moore, D.D. and Dowhan, D. 1995. Preparation and analysis of DNA, pp. 2–11. *In* Ausubel, F.W., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (eds.), Current protocols in molecular biology, Wiley, New York, USA.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Sasser, M. 1990. Identification of bacteria through fatty acid analysis, pp. 199–204. *In* Klement, Z., Rudolph, K., and Sands, D.C. (eds.), Methods in phytobacteriology, Akademiai Kaido, Budapest, Hungary.

Siddiqi, M.Z., Shah, S., Choi, K.D., Kim, S.Y., and Im, W.T. 2018. *Mesorhizobium hankyongi* sp. nov. isolated from soil of ginseng cultivating field. *Curr. Microbiol.* **75**, 1453–1459.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins., D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Velázquez, E., Igual, J.M., Willems, A., Fernández, M.P., Muñoz, E., Mateos, P.F., Abril, A., Toro, N., Normand, P., Cervantes, E., *et al.* 2001. *Mesorhizobium chacoense* sp. nov., a novel species that nodulates *Prosopis alba* in the Chaco Arido region (Argentina). *Int. J. Syst. Evol. Microbiol.* **51**, 1011–1021.

Yuan, C.G., Jiang, Z., Xiao, M., Zhou, E.M., Kim, C.J., Hozzein, W.N., Park, D.J., Zhi, X.Y., and Li, W.J. 2016. *Mesorhizobium sediminum* sp. nov., isolated from deep-sea sediment. *Int. J. Syst. Evol. Microbiol.* **66**, 4797–4802.